PHYTOCHEMICALS AND ACUTE TOXICITY OF *MORINGA OLEIFERA* BARKS IN RATS

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ABSTRACT

Objectives: The phytochemicals in *Moringa oleifera* barks were qualitatively identified in the aqueous and ethanol extracts. The acute oral toxicity of *Moringa oleifera* aqueous and ethanol extracts were done as per OECD guidelines 423. **Methods:** The barks were harvested during dry season and air dried. Extractions using ethanol and water were done. The harvested phytochemicals were qualitatively identified using standard chemicals procedures. Acute toxicity was done on 3 male and 3 female Wistar rats which were administered an initial dose of 300 mg/kg body weight followed by 2000 mg/kg & 5000 mg/kg body weight. The animals were observed for signs of convulsions, tremors, circling, depression, excitement and mortality. Body weight was recorded at 0,7th and 14th day and plasma total protein, blood sugar level, total cholesterol, SGOT & SGPT were measured to evaluate the toxicity of the extracts. **Results:** The phytochemicals identified were steroids and triterponoids, saponins, alkaloids, and carbohydrates. No abnormal sign of symptoms were observed in any of the animal fed with aqueous and ethanol extracts at the dose rate of 300 mg/kg body weight, 2000 mg/kg body weight & 5000 mg/kg body weight. No mortality was observed in any of the animals. **Conclusion:** *M. oleifera* barks, ethanol and aqueous extracts contain steroids and triterpenoids, saponins, alkaloids and carbohydrates. The administration of *M. oleifera* ethanolic and water extracts are safest & has no adverse effect on growth related and biochemical parameters indicating its safety.

KEY WORDS: *Moringa oleifera* barks; phytochemicals; acute toxicity; rats

1. INTRODUCTION

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan[1,2]. This rapidly-growing tree (also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, saijhan, sajna or Ben oil tree), was utilized by the ancient Romans, Greeks and Egyptians; it is now widely cultivated and has become naturalized in many locations in the tropics.

The scientific classification of *Moringa oleifera* shows that it comes from Kingdom: Plantae, Division: Magnoliphyta, Class: Magnoliopsida, Order: Brassicales, Family: Moringaceae, Genus: Moringa, Species: M. *oleifera*[3], which is at the moment distributed all over the world[4]**.** Almost all the parts of this plant: root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the

indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastro-intestinal, hematological and hepato-renal disorders.[5-8]

Phytochemicals are, in the strictest sense of the word, chemicals produced by plants. An examination of the phytochemicals of Moringa species affords the opportunity to examine a range of fairly unique compounds. Many of them are strong antioxidants, effective antimicrobials, possess substantial anti-carcinogenic and anti-mutagenic properties[9-11]. They are also active in reducing high blood pressure[12, 13].

Because of its several nutritional and pharmacological applications *M. oleifera* has gained popularity in many communities. Despite wide use the barks, the phytochemicals and toxicity profile are not well documented. This study set out to determine the phytochemicals and acute toxicity of *M. oleifera* barks in rats.

2. MATERIALS AND METHODS

2.1 Plant collection and extracts preparation: *M. oleifera* barks were harvested during the dry season from trees grown in Surat region of Gujarat state . The family and species of *M. oleifera* were confirmed by Hemchandra North Gujarat University, Patan and barks were kept in the University Herbarium. *M. oleifera* barks were air-dried at room temperature in the Department of Pharmacology until constant weight was attained. They were kept away from direct sun light to avoid destroying active compounds. They were then pounded to powder using metallic motor and pestle to ease the extraction of active compounds.

2.2 Extraction process: The shade dried coarsely powdered bark was extracted in a Soxhlet using ethanol and water till the complete extraction occurs. These extracts obtained in different batches were mixed up and preserved for the purpose of further studies.

2.3 Qualitative tests on the ethanol and water bark extracts: The qualitative methods already established to test for classes of compounds in plant extracts[14] were used. The substances that were tested for included: alkaloids, steroids and triterpenoids, tannins, carbohydrates, flavones, saponins, amino acids and proteins. The ethanol and water extracts were used to determine the compounds.

Hydrochloric acid, Dragendoff's reagent and Meyer's reagent were used; presence of yellowish precipitate indicated the presence of alkaloids. To detect the presence of steroids, triterpenoids, acetic anhydride, Chloroform and concentrated sulphuric acid were used, a brown-red ring at the interface between the two liquids and a green supernatant indicated their presence.

Gelatin solution, lead acetate and ferric chloride were used to indicate the presence of tannins. A white precipitate, blackish blue color and yellow precipitate indicated the presence of tannins.

Foam formation appearing after shaking for 15 min a test tube containing 1 mg Dimethylsulfoxide, ethanol and distilled water indicated presence of saponins. One milligram of dry extract was dissolved in 1 ml of methanol at 50°C, metallic magnesium and 4 to 5 drops of concentrated hydrochloric acid added. A red or orange color indicates the presence of flavonoids. Treated the extracts with a few drops of Molisch's reagent (Solution of α-napthol in alcohol) and 2 ml of conc. H_2SO_4 acid slowly through the sides of the test tube,

violet ring is formed at the junction of the two layers indicated presence of carbohydrates.

When extracts were treated with Millon's reagent, Ninhydrin reagent and Biuret reagent; white precipitate, violet colour and blue colour indicated presence of proteins and amino acids.

Chemicals in this study were used from Vidyabharti Trust College of Pharmacy, Umrakh.

2.4 Establishing LD_{50} : The ethanol and water extracts were administered orally following Organization of Economic Co-operation and Development (OECD 423) guidelines to 3 Wistar male rats and 3 female rats using limit dose in three steps (viz; exposing 3 female rats in 1st step & 3 male rats in the 2nd step to a dose of 300, 2000 and 5000 mg/kg body weight) while distilled water was given to the other group of rats as a control[15]. Further three male rats were taken up and weighed and marked for identification and same procedure was repeated. Following administration of single dose of ethanol and water extracts animals were observed for the clinical symptoms for 30 minutes, at hourly intervals for next 24 hours and thereafter for total 14 days. The animals were observed for signs of convulsions, tremors, circling, depression, excitement and mortality. Body weight was recorded at 0, 7th and 14th day and plasma total protein, blood sugar level, total cholesterol, SGOT & SGPT were measured to evaluate the toxicity of the extracts. All the animals are terminally sacrificed for gross necropsy findings.

2.5 Ethical clearance: In relation to the use of laboratory animals, the protocol used in this study was approved by the Institutional Animal Ethics Committee, Vidyabharti Trust College of Pharmacy, Umrakh (VBT/IAEC/10/12/32). The rats were kept in rat cages and fed on commercial rat pellets and allowed to freely access tap water in bottles at reassure. They enjoyed 12 hours of light and 12 hours of dark cycle.

2.6 Statistical Analysis: Results were expressed as mean ± standard error of mean (S.E.M.). Statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc Dunnett test. P values less than 0.05 were considered significant.

3. RESULTS

The phytochemical screening of aqueous and ethanol barks extracts of *M. oleifera* reviled that ethanol extracted more phytochemicals than water. None of the solvents extracted flavonoids, tannins and proteins from the bark powder (Table 1). The table also shows that all the two solvents were able to extract Steroids, saponins, carbohydrates and alkaloids with ethanol giving the strongest response.

3.1 Ethanol extract: Ethanol was able to extract steroids and triterponoids, saponins, alkaloids and carbohydrates. The greatest response for ethanol was observed in carbohydrate, alkaloids plus saponins.

3.2 Water extract: Water was able to extract saponins, carbohydrates, and alkaloids. The greatest response for that of water was in carbohydrates and alkaloids.

3.3 Lethal dose 50 (LD_{50}): All the animals were carefully observed for development of any toxic signs or symptoms at different time intervals of 0, 30 minutes, 1, 2, 4, 6, 8, 12 hrs and then daily for period of 14 days. No abnormal sign of symptoms were observed in any of the animal fed with ethanol and water extracts at the dose rate of 300 mg/kg body weight, 2000 mg/kg body weight and 5000 mg/kg body weight. No mortality was observed in any animal

indicating its safety. Hence, from the present study it can be concluded that the *M. oleifera* extracts is nontoxic at the limit dose of 5000 mg/kg body weight. No adverse effect was seen on the body weight and internal organ weight when compared with control group (Table 2 and 3) and no significant changes in the biochemical parameters from those of normal values of these parameters were observed as compared to control, indicating no adverse effect on the liver at experimental dose rate (Table 4). No adverse effects, was seen even at a higher limit dose of 5000 mg/kg. Clinical examination of all the rats were normal and necropsy findings does not show any remarkable findings.

CONCLUSION

M. oleifera barks, ethanol and aqueous extracts contain steroids and triterpenoids, saponins, alkaloids and carbohydrates. The administration of *M. oleifera* ethanolic and water extracts are safest & has no adverse effect on growth related and biochemical parameters. It is also inferred that these extracts are safe at a higher limit dose, belongs to class 5 or unclassified substances as per Globally Harmonized Classified System (GHC) for chemical substances indicative of very high LD_{50} value.

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Table 1: Phytochemicals present in the *M. oleifera* barks

Phytochemicals	Ethanol extract	Aqueous extract
Steroids and triterpenoids	+	-
Saponins	+++	+
Flavonoids	-	-
Carbohydrates	+++	++
Alkaloids	+++	++
Tannins and phenolic	-	-
Proteins and amino acids	-	-

-: not detected; +: present in low concentration; ++: present in moderate concentration; +++ present in high concentrations.

Table 2: Body weight of rats in acute toxicity study of extracts of bark of *M. oleifera*

	Body weight (g)			
	Day 0	**Day 7**	**Day 14**	**Weight gain on day 14**
Female				
Control	146.3±1.85	153.07±2.72	147.03±2.60	41.20±1.62
M. oleifera 5000 mg/kg Ethanolic Extract	155.7±2.33	155.00±3.78	150.07±4.05	49.60±3.87
M. oleifera 5000 mg/kg Aqueous Extract	152.0±3.60	157.30±2.33	147.7±3.84	47.88±2.76
Male				
Control	152.3±1.45	150.0±1.15	155.7±0.66	86.00±6.60
M. oleifera 5000 mg/kg Ethanolic Extract	156.7±2.40	161.3±2.90	164.3±3.28	81.60±2.86
M. oleifera 5000 mg/kg Aqueous Extract	159.7±3.18	164.3±4.25	169.7±6.06	84.41±3.18

Values are expressed as mean ± S.E.M., n = 3.
There were no significant differences at $p<0.05$.

Table 3: Organ weight of rats in acute toxicity study of extracts of bark of *M. oleifera*

	Organ body weight (g)		
	Control	**M. oleifera 5000 mg/kg Ethanolic Extract**	**M. oleifera 5000 mg/kg Aqueous Extract**
Female			
Lung	1.05±0.06	1.16±0.09	1.10±0.08
Heart	0.78±0.04	0.87±0.06	0.89±0.07
Liver	7.60±0.38	6.83±0.30	6.76±0.35
Spleen	0.52±0.03	0.59±0.05	0.56±0.05
Kidney	0.81±0.04	0.76±0.03	0.80±0.04
Ovary	0.06±0.00	0.07±0.00	0.06±0.00
Male			
Lung	1.15±0.05	1.17±0.06	1.17±0.06
Heart	0.98±0.04	0.93±0.04	0.90±0.04
Liver	8.60±0.87	7.81±0.48	7.98±0.57
Spleen	0.74±0.05	0.70±0.03	0.75±0.06
Kidney	1.11±0.04	0.98±0.02	0.90±0.02
Testis	1.86±0.07	1.27±0.03	1.21±0.02

Values are expressed as mean ± S.E.M., n = 3.
There were no significant differences at p<0.05.

Table 4: **Effects of ethanolic and aqueous extracts on rat's blood parameters after oral administration (5000 mg/kg body weight)**

Sr. No.	**Biochemical parameters**	**Control**		**Ethanolic extract**		**Aqueous extract**	
		7 Day	**14 day**	**7 Day**	**14 day**	**7 Day**	**14 day**
01	Total protein (g/dl)	6.60 ± 0.09	5.97 ± 0.11	6.45 ± 0.10	6.07 ± 0.13	6.40 ± 0.10	6.0± 0.02
02	Blood sugar (mg/dl)	62.00 ± 1.08	61.75 ± 4.00	61.50 ± 0.64	64.75 ± 1.65	62.45 ± 1.02	63.19 ± 1.53
03	Total cholesterol (mg/dl)	65.75 ± 2.83	53.50 ± 4.62	65.75 ± 2.83	56.75 ± 3.54	62.37 ± 2.10	52.48 ± 3.94
04	SGOT (IU/L)	47.75 ± 8.75	44.25 ± 1.93	53.24 ± 7.38	50.00 ± 4.08	50.62± 4.10	46.39 ± 2.78
05	SGPT (IU/L)	43.25 ± 5.80	50.75 ± 2.17	54.50 ± 10.88	52.75 ± 4.42	51.44 ± 9.16	47.31 ± 2.09

Values are expressed as mean ± S.E.M., n = 3.
There were no significant differences at p<0.05.